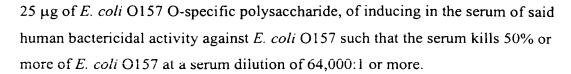
CLAIMS

We claim:

- 1. A conjugate molecule comprising the *E. coli* O157 O-specific polysaccharide, covalently bound to a carrier selected from the group consisting of: the B subunit of Shiga toxin 1, the B subunit of Shiga toxin 2, a non-toxic mutant Shiga toxin 1 holotoxin, and a non-toxic mutant Shiga toxin 2 holotoxin.
- 2. The conjugate molecule of claim 1 wherein the *E. coli* O157 O-specific polysaccharide is covalently bound to the B subunit of Shiga toxin 1 by means of a dicarboxylic acid dihydrazide linker.
- 3. The conjugate molecule of claim 2 wherein the dicarboxylic acid dihydrazide is adipic acid dihydrazide.
- 4. The conjugate molecule of claim 1 wherein the *E. coli* O157 O-specific polysaccharide is covalently bound to the B subunit of Shiga toxin 1 by a process which comprises the steps of
 - (a) cyanation of the *E. coli* O157 O-specific polysaccharide with a cyanylation reagent; and
 - (b) reaction of the B subunit of Shiga toxin 1 with the resulting cyanated *E. coli* O157 O-specific polysaccharide.
- 5. The conjugate molecule of claim 4 wherein the cyanylation reagent is 1-cyano-4-(N,N-dimethylamino)pyridinium tetrafluoroborate.
- 6. A pharmaceutical composition comprising a conjugate molecule of any one of claims 1-5, further comprising a pharmaceutically acceptable carrier.
- 7. The pharmaceutical composition of claim 6, further comprising an adjuvant.
- 8. The pharmaceutical composition of claim 6, wherein the composition is capable, upon injection into a mouse of an amount of said composition containing 2.5 μg of *E. coli* O157 O-specific polysaccharide, of inducing in the serum of said mouse antibodies which neutralize the toxicity of Stx1 toward HeLa cells.

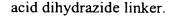
- 9. The pharmaceutical composition of claim 6, wherein the composition is capable, upon injection into a mouse of an amount of said composition containing 2.5 μg of E. coli O157 O-specific polysaccharide, of inducing in the serum of said mouse antibodies which neutralize the toxicity of Stx1 toward HeLa cells.
- 10. A vaccine composition comprising a conjugate molecule, said conjugate molecule comprising the *E. coli* O157 O-specific polysaccharide covalently bound to a carrier protein, in a pharmaceutically acceptable carrier.
- 11. The vaccine composition of claim 10, wherein the carrier protein is selected from the group consisting of native or mutant forms of: tetanus toxoid, diptheria toxoid, pertussis toxoid, : the B subunit of Shiga toxin 1, the B subunit of Shiga toxin 2, a non-toxic mutant Shiga toxin 1 holotoxin, a non-toxic mutant Shiga toxin 2 holotoxin, Clostridium perfringens toxoid, Clostridium welchii exotoxin C. Pseudomonas aeruginosa recombinant exoprotein A, hepatitis B surface antigen, hepatitis B core antigen, and bovine serum albumin.
- 12. The vaccine composition of claim 11, wherein the carrier protein is selected from the group consisting of *Clostridium welchii* exotoxin C, *Pseudomonas aeruginosa* recombinant exoprotein A, B subunit of Shiga toxin 1, and bovine serum albumin.
- 13. The vaccine composition of any one of claims 10 12, wherein the composition is capable, upon injection into a human of an amount of said composition containing 25 μg of *E. coli* O157 O-specific polysaccharide, of inducing in the serum of said human bactericidal activity against *E. coli* O157 such that the serum kills 50% or more of *E. coli* O157 at a serum dilution of 1300:1 or more.
- 14. The vaccine composition of any one of claims 10 12, wherein the composition is capable, upon injection into a human of an amount of said composition containing 25 μg of *E. coli* O157 O-specific polysaccharide, of inducing in the serum of said human bactericidal activity against *E. coli* O157 such that the serum kills 50% or more of *E. coli* O157 at a serum dilution of 32,000:1 or more.
- 15. The vaccine composition of any one of claims 10 12, wherein the composition is capable, upon injection into a human of an amount of said composition containing



- 16. The vaccine composition of any one of claims 10 12, wherein the composition is capable, upon injection into a human of an amount of said composition containing 25 μg of *E. coli* O157 O-specific polysaccharide, of inducing in the serum of said human at least a 50-fold rise in IgG which immunoreacts with *E. coli* O157 LPS, when said IgG is measured 4 weeks post injection.
- 17. The vaccine composition of any one of claims 10 12, wherein the composition is capable, upon injection into a human of an amount of said composition containing 25 μg of *E. coli* O157 O-specific polysaccharide, of inducing in the serum of said human at least a 60-fold rise in IgG which immunoreacts with *E. coli* O157 LPS, when said IgG is measured 26 weeks post injection.
- 18. The vaccine composition of any one of claims 10 12, further comprising an adjuvant.
- 19. A method of inducing in a mammal serum antibodies that are bacteriostatic or bactericidal to *E. coli* O157, comprising administering to said mammal, in a physiologically acceptable carrier, a conjugate molecule of any one of claims 1-5.
- 20. The method of claim 18 wherein said conjugate molecule is administered at a dose of about 5 micrograms to about 50 micrograms of *E. coli* O157 O-specific polysaccharide.
- 21. The method of claim 18 wherein the antibodies protect the mammal against infection by *E. coli* O157.
- 22. A composition comprising antibodies which are immunoreactive with *E. coli* O157 O-specific polysaccharide.
- 23. The composition of claim 22, further comprising antibodies which are immunoreactive with the B subunit of Shiga toxin 1.
- 24. The composition of claim 22, wherein the composition is chosen from the group

BEST AVAILABLE COPY

- consisting of mammalian plasma, mammalian serum, and mammalian gamma globulin fraction.
- 25. The composition of claim 23, wherein the composition is chosen from the group consisting of mammalian plasma, mammalian serum, and mammalian immunoglobulin fraction.
- 26. An antibody which is immunoreactive with *E. coli* O157 O-specific polysaccharide.
- 27. A method of passively immunizing a mammal against *E. coli* O157, comprising administering to said mammal an immunologically sufficient amount of a composition according to any one of claims 22 25.
- 28. The method of claim 27 wherein the antibody is administered at a dose in the range of from about 1 mg/kg to about 10 mg/kg body weight of the mammal.
- 29. The method of claim 28 wherein the mammal is a human.
- 30. A method for vaccinating a mammal against *E. coli* O157 infection, comprising administering to the human an immunizing amount of a composition according to claim 6.
- 31. The method of claim 30 wherein the mammal is a human.
- 32. A method for vaccinating a mammal against *E. coli* O157 infection, comprising administering to the human an immunizing amount of a vaccine composition according to any one of claims 10 12.
- 33. The method of claim32 wherein the mammal is a human.
- 34. A conjugate molecule comprising an O-specific polysaccharide, covalently bound to the B subunit of Shiga toxin 1 or Shiga toxin 2, or to a non-toxic mutant Shiga holotoxin, wherein the O-specific polysaccharide is an O-specific polysaccharide of a bacterium chosen from the group consisting of: *E. coli* O157, *E. coli* O111, *E. coli* O17, *E. coli* O26, and *Shigella dyenteriae*.
- 35. The conjugate molecule of claim 34 wherein the O-specific polysaccharide is covalently bound to the B subunit of Shiga toxin 1 by means of a dicarboxylic



- 36. The conjugate molecule of claim 35 wherein the dicarboxylic acid dihydrazide is adipic acid dihydrazide.
- 37. The conjugate molecule of claim 36 wherein the O-specific polysaccharide is covalently bound to the B subunit of Shiga toxin 1 by a process which comprises the steps of
 - (a) cyanation of the O-specific polysaccharide with a cyanylation reagent; and
 - (b) reaction of the B subunit of Shiga toxin 1 with the resulting cyanated O-specific polysaccharide.
- 38. The conjugate molecule of claim 37 wherein the cyanylation reagent is 1-cyano-4-(N,N-dimethylamino)pyridinium tetrafluoroborate.
- 39. A pharmaceutical composition comprising a conjugate molecule of any one of claims 34-37 further comprising a pharmaceutically acceptable carrier.
- 40. A composition coprising antibodies which are immunoreactive with Shiga toxin 1 or Shiga toxin 2.
- 41. A method of administering a composition of claim 40 to a mammal in an immunologically sufficient amount.